Structural and Functional Insights into the Human Pneumoviruses Glycoprotein Domain: Key Components for Vaccine Development

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Bachelor's Degree in Microbiology Final Project

1 Introduction to Human Pneumoviruses

Human orthopneumovirus and Human metapneumovirus

The *Human orthopneumovirus* (HRSV) and the *Human metapneumovirus* (HMPV) (1) are the major causative agents of acute respiratory infections mainly within the paediatric population (<2 years of age). Both belong to the *Pneumoviridae* family and share genomic, structural and functional similarities **[Figure 1]**. Both show global distribution, with a clear pattern of seasonality extending from winter to spring months.

OBJECTIVES: the purpose of this study is to review HRSV and HMPV viral transmembrane proteins biology, structure and function. In addition, the glycoproteins antigenicity, viral strategies of immune evasion and the current and futue challenges of vaccine development wants to be described.

METHODOLOGY: for the elaboration of this project several articles, reports and reviews have been consulted. Unless specifically mentioned, figures were elaborated with Microsoft® PowerPoint or PyMol Molecular Graphics System v2.1.

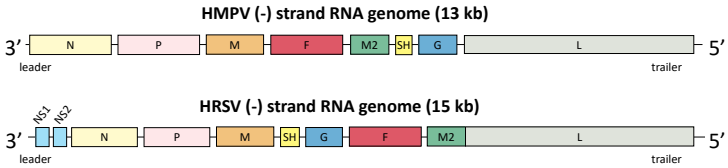

Figure 1 – HRSV and HMPV viral genome organization.
Both human pneumoviruses highly resemble each other and exhibit a linear negative-sensed (-) single stranded RNA genome of 13kb (HMPV) or 15kb (HRSV) in length. The HRSV genome has two unique non-structural genes (NS1/NS2) at the leader sequence of RNA. The nucleoprotein (N), phosphoprotein (P), Matrix (M) and polymerase (L) genes organization is shared. Differences are found in organization of the small hydrophobic (SH), Fusion (F), Attachment (G) and M2 genes.

2 Major Human Pneumoviruses Surface Glycoproteins

Which is their strcuture and function?

Initial phases of infection are directed by the attachment (G) and fusion (F) glycoproteins. The G glycoprotein targets the HSPGs and CX3CR1 cell receptors. However, it is non-essential for viral infectivity. By contrast, the F glycoprotein is essential for infectivity and suffers a major refolding event upon entry in which the Fusion Peptide (FP) found at the F_1 subunit is extruded promoting membrane fusion. Moreover, viral attachment, in absence of the G protein, might be carried out by F protein. While the F glycoprotein remains highly conserved in sequence, the G glycoprotein is highly variable, mostly in its mucin-like domains. A soluble G protein (sG) acting as an antibody-decoy has also been identified.

Fusion Glycoprotein (F)

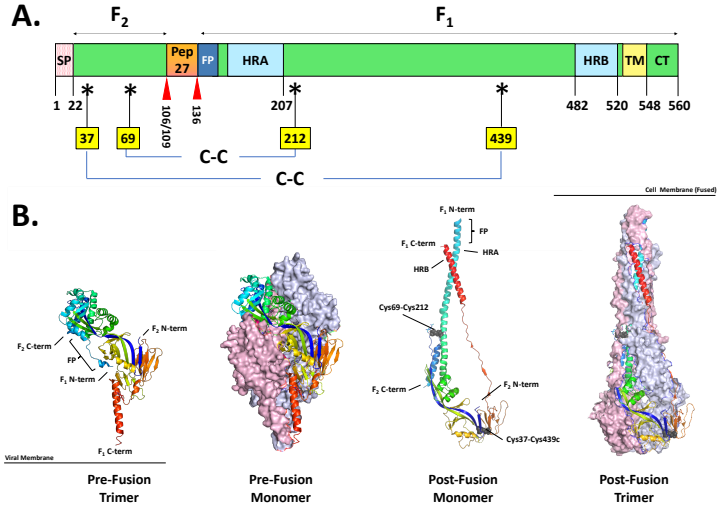

Figure 2 – Diagram of the HRSV-F gene and structural representation of the HRSV-F glycoprotein in pre- and post-fusion conformation.
(A) The signal peptide (SP) induces the translocation of the F protein towards the cell membrane. Furin-protease cleavage sites are indicated in red, surrounding Pep27. F_2 and F_1 subunits are formed and linked by disulfide bonds (C-C) between cysteines (*), tridimensionaly represented by grey bubbles. **(B)** Representation of the HRSV pre-fusion (left - PDB: 5TOJ) and post-fusion (right - PDB: 3RRR) monomers and trimers has been assessed using Pymol Molecular Graphics System v2.1 (Schrödinger, LLC, NY, USA). Major refolding of the pre-fusion conformation into the post-fusion state occurs in which the F_1 C-term and F_1 N-term regions are extruded, permitting the insertion of the fusion peptide (FP) into the target cell, enabling fusion. HR= Heptad Repeat TM= transmembrane domain. CT= cytoplasmic domain.

Attachment Glycoprotein (G)

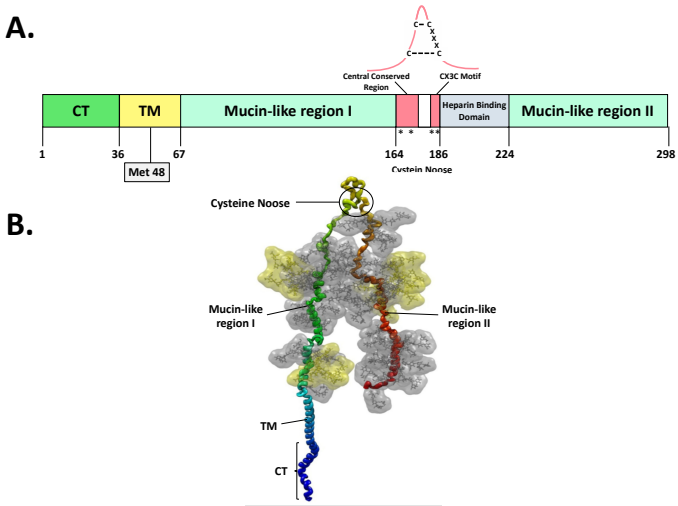

Figure 3 – Diagram of HRSV-G gene and structural representation of the HRSV-G glycoprotein.
(A) Three main unglycosylated regions consisting of cytoplasmic, transmembrane and the central conserved domains are found in HRSV-G protein. Two highly-variable and glycosylated mucin-like regions (I - II), surround the central conserved domain that forms an unglycosylated cysteine noose. Cysteines involved in the formation of it are represented by the asterisk (*) symbol. The heparin binding domain and the CXC3-motif bind to the cell receptors heparin sulfate proteoglycans and CX3CR1 respectively. **(B)** Complex *N*-linked (yellow) and simple the *O*-linked (grey) glycans decorate the protein, illustrating the size of the glycosylated G form in comparison with its backbone sequence. Methionine-48 (Met48) serves as the start codon for the sG protein. Tridimensional representation of the HRSV-G protein was performed by McLellan *et al* 2013 (2).

3 Antigenicity of Fusion glycoproteins

Describing antigènic sites I, II, III and Ø.

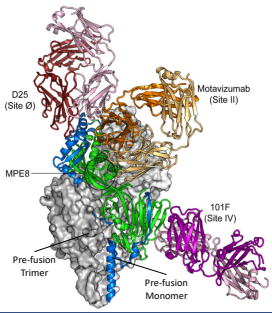

Figure 4 – Tridimensional representation of neutralizing antibodies bound to antigenic sites in HRSV-F glycoprotein.
Antigenic sites I, II, IV and Ø are found on HRSV-F. Sites II and IV are also present in the post-fusion conformation whereas antigenic site Ø (D25 - red) remains exclusive of the pre-fusion state. The strain-dependent antigenic site I is situated in the F_1 subunit. Antigenic site II is the binding site of motavizumab (orange). The MPE8 antibody (green) cross-competes with motavizumab for binding to pre-fusion HRSV-F state, partially overlapping antigenic site II. MPE8 site is highly-conserved and can neutralize both HRSV and HMPV. Finally, 101F antibody (purple) binds the antigenic site IV. These neutralizing antibodies prevent the fusion of viral and cell membranes. Illustration performed by McLellan *et al.* 2015 (3).

4 Immune Evasion

Viral Shielding

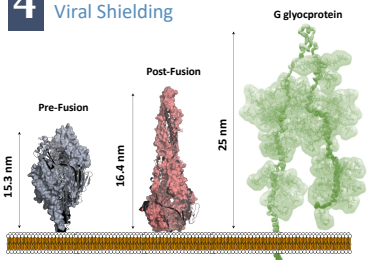

Figure 5 – Antigenic Shielding explained by differences of size and volume between HRSV glycoproteins.
The G protein due to its massive surface glycosylation, which confers great volume (25nm height) and mass to the protein, hinders the neutralizing antibodies binding to the pre-fusion (13nm) and post-fusion (17nm) conformations, both smaller in size. It exhibits a shielding function preventing the recognition of the F glycoprotein epitopes by neutralizing antibodies. This evasive mechanism may be an important drawback for developing vaccines against these pneumoviruses, as the antibodies generated after vaccination may not reach its target nor neutralize the virus .

5 Vaccine Strategies

Different strategies towards a common end.

Target populations who might benefit from a HRSV vaccine are: infants <6 months, children >6 months, pregnant women and the elderly (>65 years). The main neutralizing determinant on the HRSV virion is the HRSV-F glycoprotein which is also highly conserved in sequence, thus most vaccine approaches are based on it. Live-attenuated vaccines are also being developed but, ensuring the balance between the virulence of the vaccine strain and the immunogenicity elicited is an arduous task and often the innocuity of the vaccine is questioned. No vaccines are currently available for HRSV nor HMPV.

		Phase I	Phase II		Phase III	Marketed
A	Vaccines	**40 ongoing studies** • North America: 34 • Europe: 6 • South America: 2 • Africa: 2 • Middle East: 1 • Australia: 1	Bavarian Nordic MVA-BN-RSV GlaxoSmithKlien GSK3003891A GlaxoSmithKlien GSK3389245A	Janssen Vaccines & Prevention B.V. Ad26.RSV.preF MedImmune MEDI-534 MedImmune MEDI-559	Novavax RSV-F Vaccine	
B	Immunoglobulins	**10 ongoing studies**	**3 ongoing studies**		Regenereon REGN-222	Palivizumab
C	Antiviral	**13 ongoing studies**	**3 ongoing studies**			Ribavirin

Figure 6 – Overview of HRSV treatment development. General overview of HRSV vaccine (A), immunoglobulins (B) and antiviral (C) treatment development. Company name and product are given and classified by development stage (phase I-III and marketed). Only, palivizumab and the antiviral drug ribavirin have been licensed for administration. Image up to date through May 2018. Data collected from U.S. National Library of Medicine - Clinical Trials website (www.clinicaltrials.gov).

6 Conclusions

Final overview

Overall, since F protein is relatively conserved in sequence, its surface location, its essentiality for viral entry, its immunogenicity and, the fact that has the ability to elicit a protective immunity; make the F protein an ideal target for neutralizing antibodies and a promising candidate for vaccine development. By contrast, the G protein is another major antibody target, but due to its high variability and glycan decoration makes it not a suitable candidate for prophylactic treatment development. Nonetheless, several promising vaccines are currently being developed and one or both of the main glycoproteins are included in most vaccine modalities.

Relevant References
[1.] van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RAM, Osterhaus ADME. 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 7:719–724.
[2.] McLellan JS, Ray WC, Peeples ME. 2013. Structure and Function of Respiratory Syncytial Virus Surface Glycoproteins.
[3.] McLellan JS. 2015. Neutralizing epitopes on the respiratory syncytial virus fusion glycoprotein. Curr Opin Virol.

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